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Review Article

Regulation of mTORC1 by amino acids in mammalian cells: A general picture of recent advances

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ABSTRACT

The mechanistic target of rapamycin complex 1 (mTORC1) integrates various types of signal inputs, such as energy, growth factors, and amino acids to regulate cell growth and proliferation mainly through the 2 direct downstream targets, eukaryotic translation initiation factor 4E-binding protein 1 (4EBP1) and ribosomal protein S6 kinase 1 (S6K1). Most of the signal arms upstream of mTORC1 including energy status, stress signals, and growth factors converge on the tuberous sclerosis complex (TSC) – Ras homologue enriched in brain (Rheb) axis. Amino acids, however, are distinct from other signals and modulate mTORC1 using a unique pathway. In recent years, the transmission mechanism of amino acid signals upstream of mTORC1 has been gradually elucidated, and some sensors or signal transmission pathways for individual amino acids have also been discovered. With the help of these findings, we propose a general picture of recent advances, which demonstrates that various amino acids from lysosomes, cytoplasm, and Golgi are sensed by their respective sensors. These signals converge on mTORC1 and form a huge and complicated signal network with multiple synergies, antagonisms, and feedback mechanisms.

1. Introduction

The mammalian or mechanistic target of rapamycin (mTOR) as suggested by Huang and Fingar (2014) is an atypical serine (Ser)/threonine (Thr) kinase that is quite conserved through evolution (Jewell et al., 2013). The kinase exists in 2 different protein complexes in mammalian cells named mTOR complex 1 (mTORC1) and complex 2 (mTORC2) respectively. The activity of mTORC1 is regulated by various cues including intracellular energy status, extracellular growth factors such as insulin, stress factors, and the availability of amino acids (AA). The integrated responses in mTORC1 activity plays a central role in the regulation of cell growth and proliferation by affecting various intracellular processes including autophagy and overall protein synthesis (Bhaskar and Hay, 2007). Unlike other factors that affect mTORC1 activity, the transduction pathway of AA signal has not been fully elucidated as yet but great progress in understanding this pathway has been made in recent years. The aims of the present review were to describe a general picture of recent advances in this area.

2. mTOR and mTORC1 signaling pathway

2.1. mTOR

mTOR was the binding target of rapamycin identified in mammalian cells. In 1975, Vézina et al. (1975) isolated a strain of *Streptomyces hygroscopicus* in the soil of the Easter Island that can secrete an antifungal macrolide named rapamycin (Abraham and Wiederrecht, 1996). Clinical use of rapamycin as an antifungal drug was soon stopped due to its immunosuppressive and antiproliferative effects. Later research interest in rapamycin was mainly on the mechanisms of its immunosuppressive and antiproliferative effects. It was found that rapamycin caused binding

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to FK506-binding protein 12 kDa (FKBP12) and other proteins after entering fungi cells to depress cell proliferation (Yang and Guan, 2007). The discovery of anti-rapamycin mutants of *Saccharomyces cerevisiae* led to the identification of the 2 wild genes encoding 2 approximate 300 kDa rapamycin binding proteins named TOR1 and TOR2 respectively (Heitman et al., 1991). The homologue of rapamycin binding protein in mammalian cells (mTOR) was soon identified but was encoded by only one gene (Brown et al., 1994; Chiu et al.,1994; Sabatini et al., 1994). Further studies revealed that mTOR is an atypical Ser/Thr protein kinase belonging to the phosphatidylinositol 3-kinase-related protein kinase (PIKK) family, which exists in 2 different protein complexes named mTORC1 and mTORC2 respectively in mammalian cells with different function and structure (Schmelzle and Hall, 2000; Saxton and Sabatini, 2017).

As an important functional complex in mammalian cells, mTORC1 consists of mTOR, regulatory-associated protein of mTOR (Raptor), 40 kDa Pro-rich AKT substrate (PRAS40, also known as AKT1S1), DEP domain-containing mTOR-interacting protein (Deptor), and mammalian lethal with SEC13 protein 8 (mLST8, also known as GβL) (Fig. 1A). In addition, some reports regard FKBP38 as a bona fide component of mTORC1 (Yoon and Choi, 2016). As a scaffold protein mTOR, Raptor can bind substrates and modulate the phosphorylation of mTOR (Yonezawa et al., 2004). PRSA40 combines with Raptor and regulates mTORC1 activity through protein kinase B (PKB, also known as Akt) (Sancak et al., 2007). Deptor can interact with mTOR and inhibit its kinase activity, and then regulate the function of the endoplasmic reticulum (ER) through its transcription (Catena and Fanciulli, 2017). The up-regulation of mLST8 can positively regulate mTORC1 activity, but its role in different cells may have different degrees of the opposite function (Kakumoto et al., 2015). Regardless, this result suggests that mLST8 may not be necessary for mTORC1 (Guertin et al., 2006).

Due to its insensitivity to rapamycin and uncertain function, there has been limited knowledge of mTORC2. Since entering the 21st century, new findings have contributed to an understanding of the structure and functional mechanism of mTORC2 which are summarized by Yang and Guan (2007). The composition of mTORC2 is different from mTORC1, in which rapamycin-insensitive companion of mTOR (Rictor) (Sarbassov et al., 2004) and Sin1 (also known as Mip1) (Jacinto et al., 2006) are defined as essential subunits of mTORC2, but not mTORC1 (Fig. 1B). Rictor and Sin are homologues of the TOR2 subunits AVO3 and AVO1 in yeast, respectively, which means that mTORC2 is evolutionarily conserved, just like mTORC1. mTORC2 also includes mLST8 and Deptor, consistent with mTORC1, whereas mLST8 is necessary to maintain the function of mTORC2, inconsistent with mTORC1 (Guertin et al., 2006) (Fig. 1B). The knockout of Rictor or Sin1 leads to a decrease in the protein levels of other mTORC2 components, implying the importance of both in maintaining the stability of the mTORC2 complex (Yang et al., 2006). mTORC2 was initially thought to function upstream of Rho GTPases to regulate the actin cytoskeleton (Jacinto et al., 2004). Subsequently, mTORC2 was identified as having a new subunit PRR5 (Woo et al., 2007), which can regulate Akt activity together with Sin1. Due to this, mTORC2 has been considered as a potential upstream signal node for mTORC1 (Yang and Guan, 2007) (Fig. 1B).

2.2. The mTORC1 signaling pathway

As a key integrator of cues, including nutrient and growth factor availability as well as stress, mTORC1 responds to multiple variations in cells and regulates various cell activities (Fig. 2). Following the evolution from single-celled organisms to advanced mammals, the signal-network upstream of mTORC1 has become more complex and diverse. These changes have helped animals adapt to harsh living environments and meet different functional needs in their various respective organs and tissues. One classical regulator of mTORC1 is Ras homologue enriched in brain (Rheb), a small GTPase, whose activity is determined by its guanine nucleotide state. Some studies have shown that Rheb can respond to growth factor stimuli and available nutrients, antagonizing the mTOR endogenous inhibitor, FKBP38, in a GTP-dependent manner (Bai et al., 2007), and bind to the carboxyl terminal of the mTOR catalytic domain to regulate its kinase activity using phospholipase D1 (PLD1) (Long et al., 2005a; Sancak et al., 2007; Sun et al., 2008). The upstream target of Rheb is the tuberous sclerosis complex (TSC), which includes TSC1 (also known as hamartin) and TSC2 (also known as tuberin), and has recently been proposed to contain a third subunit, TBC1D7 (Dibble et al., 2012). TSC2 is a component of the TSC complex that mainly regulates the mTORC1 signal. As the GTPase-activating protein (GAP) of the Rheb, it induces Rheb to lose the function of activating mTORC1 by converting the GTP bound to Rheb into GDP (Inoki et al., 2003a). TSC1 is responsible for stabilizing the structure and function of TSC2 (Chong-Kopera et al., 2006). The TSC is mainly regulated by growth factors and energy signals. By sensing glucose and ATP levels, Thr1227 and Ser1345 on TSC2 are phosphorylated by AMP-activated protein kinase (AMPK) (Inoki et al., 2003b). Follow-up studies by Inoki et al. found that AMPK can also transmit signals via another evolutionarily conserved signaling pathway, the canonical Wnt pathway (Inoki et al., 2006). Raptor is also one of its downstream targets (Gwinn et al., 2008). There are also reports that the mTORC1 signaling is related to Hexokinase-II (HK-II), which is a predominant isoform in the heart, as well as adipose and skeletal muscle. In the case of glucose deprivation, HK-II binds to and inhibits mTORC1, then induces autophagy (Roberts et al., 2014). Roh and others believe that telomerase is involved in this process (Roh et al., 2018).

Fig. 1. Compositions of mTORC1 (A) and mTORC2 (B). mTORC1 = mechanistic target of rapamycin complex 1; PRAS40 = 40 kDa Pro-rich AKT substrate; Raptor = regulatory-associated protein of mTOR; mTOR = mammalian or mechanistic target of rapamycin; Deptor = DEP domain-containing mTOR-interacting protein; mLST8 = mammalian lethal with SEC13 protein 8; mTORC2 = mechanistic target of rapamycin complex 2; Sin1 = Mip1; Rictor = rapamycin-insensitive companion of mTOR.

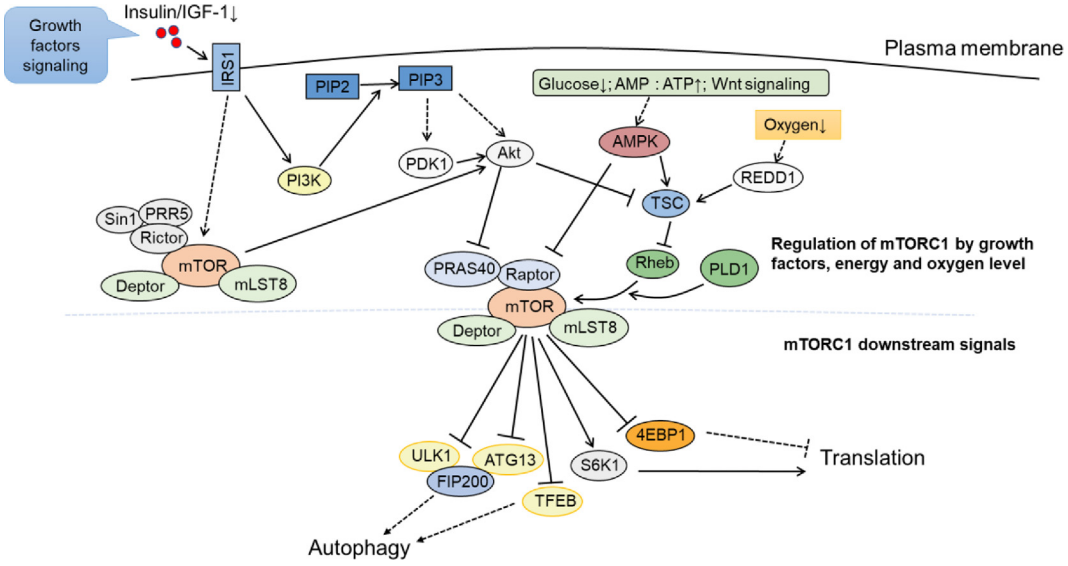

Fig. 2. Regulation of mTORC1 signaling pathway by growth factors, energy and oxygen levels. IGF-1 = insulin-like growth factor-1; PIP2 = Phosphatidylinositol-4,5-triphosphate; PIP3 = Phosphatidylinositol-3,4,5-triphosphate; Sin1 = Mip1; Rictor = rapamycin-insensitive companion of mTOR; mTOR = mammalian or mechanistic target of rapamycin; Deptor = DEP domain-containing mTOR-interacting protein; mLST8 = mammalian lethal with SEC13 protein 8; PI3K = phosphatidylinositol-3-kinase; PDK1 = 3-phosphoinositide-dependent protein kinase-1; PKD1 = phosphoinositide-dependent kinase 1; Akt = protein kinase B; AMPK = AMP-activated protein kinase; TSC = the tuberous sclerosis complex; REDD1 = transcriptional regulation of DNA damage response 1; PRAS40 = 40 kDa Pro-rich AKT substrate; Raptor = regulatory-associated protein of mTOR; Rheb: Ras homologue enriched in brain; PLD1 = phospholipase D1; ULK1 = Unc-51-like kinase 1; ATG13 = autophagy-related gene 13; FIP200 = focal adhesion kinase family-interacting protein of 200 kDa; TFEB = transcription factor EB; S6K1 = ribosomal protein S6 kinase 1; IRS1: insulin receptor substrate 1; 4EBP1 = eukaryotic translation initiation factor 4E-binding protein 1.

The reception of growth factor signals by TSC is relatively complicated and mainly depends on 2 key nodes: phosphatidylinositol-3-kinase (PI3K) and Akt. Taking insulin and insulin-like growth factor-1 (IGF-1) as an example, first, they bind to their receptors, promoting the phosphorylation of the insulin receptor substrate (IRS), which allows the latter to bind and activate PI3K with its SH2 domain (Myers et al., 1992). Activated PI3K produces Phosphatidylinositol-3,4,5-triphosphate (PIP3) and directly and/or indirectly activates Akt through 3-phosphoinositide-dependent protein kinase-1 (PDK1) (Bond, 2016). Akt is the key to the upstream signal of mTORC1, which receives growth factor signals through the PI3K pathway and stimulates mTORC1 in at least 2 independent ways. This is through either phosphorylation of multiple sites of TSC2 and/or PRAS40, or negative feedback from mTORC1 (Huang and Manning, 2009; Menon et al., 2014). Additionally, as a downstream target of mTORC2, Akt connects mTORC1 and mTORC2 in series (Huang and Manning, 2009; Yang and Guan, 2007). mTORC1 also senses the availability of oxygen, and when the intracellular oxygen concentration decreases, REDD1 (also known as RTP801/Dig2/DDIT4) is activated by transcription and signals to TSC2 (Brugarolas et al., 2004). Most inputs rely on the PI3K/Akt and TSC pathways to control mTORC1 activity, but AA signals are relatively independent (Bond, 2016; Goberdhan et al., 2016; Kim, 2009; Roccio et al., 2006). We discuss how AA regulate mTORC1 in section 3.

Recent studies have revealed some other pathways that regulate mTORC1 signal. The ubiquitin-proteasome system (UPS) involves many aspects of cellular processes, such as cellular differentiation, cell cycle progression and survival. There is some evidence which also supports the key role of ubiquitin-mediated modification in the dynamic regulation of mTORC1 signaling pathways. E3 ligase is a substrate recognition subunit that can recognize specific protein motif sequences in the substrate for binding. Ubiquitin-mediated mTORC1 signal regulation, which mostly using E3 ubiquitin ligases as main tools, can regulate mTORC1 and its downstream activities by degrading protein targets, for example, mTOR, Deptor, Akt and key proteins related to mTORC1 signaling pathway, to terminate mTORC1 signaling, regulating protein cell positioning or promoting the binding of proteins to their binding partners (Jiang et al., 2019; Yeh et al., 2018; Zhao and Sun, 2012). Some fatty acids have been reported to act as regulators of mTORC1 signaling. For example, palmitate activates mTORC1/p70S6K signaling through AMPK inhibition, phosphorylation of Raptor (Kwon and Querfurth, 2015) and enhancing the recruitment of mTOR on the lysosomal membrane (Yasuda et al., 2014), but this process seems to be accompanied by insulin resistance (Kumar and Tikoo, 2015). Besides, docosahexaenoic acid, another fatty acid, induces autophagy and promotes apoptosis of human cancer cells through p53/AMPK/mTOR signaling (Jing et al., 2011). Under stress conditions, for example, DNA damage and some toxic conditions, the tumor suppressor p53 plays an important role in maintaining cell homeostasis. Activation of p53 inhibits mTOR activity and regulates its downstream targets, including autophagy, which involves AMPK activation and requires TSC complex, both of which respond to energy deprivation (Feng et al., 2005). p53 and mTORC1 signaling machineries can cross-talk and coordinately regulate cell growth, proliferation, and death. For a detailed explanation, please refer to Budanov's and Feng's reviews (Budanov, 2011; Feng, 2010). Besides, microRNA, a class of short (18 to 22 nucleotides) noncoding RNA that regulate the expression of a wide variety of genes, have been reported to control the expression of the protein components of mTORC1 as well as mTORC2 through epigenetic regulation (Zhang et al., 2017).

The main functions of mTORC1 in mammalian cells are promoting protein synthesis, stimulating ribosome, lipid and nucleotide biosynthesis and inhibiting autophagy (Kim et al., 2013; Ma and Blenis, 2009). Surprisingly, only 2 direct downstream targets of mTORC1, ribosomal protein S6 kinase 1 (S6K1) and eukaryotic translation initiation factor 4E-binding protein 1 (4EBP1), have been identified (Avruch et al., 2009) (Fig. 2). Both 4EBP1 and S6K1 are proteins that modulate translation initiation. These kinds of proteins are usually the main regulatory hubs of cellular translation control mechanisms (Gingras et al.,1999). The translation initiation

of mRNA requires the loading of the eukaryotic translation initiation factor 4F (eIF4F) complex at the 5′ cap structure of the nascent mRNA, which is composed of eukaryotic translation initiation factors 4E (eIF4E), 4G (eIF4G) and 4A (eIF4A) (Sonenberg et al., 2000). 4EBP1 binds competitively to eIF4E and inhibits its interaction with eIF4G. Activated mTORC1 can phosphorylate 4EBP1, thus dissociating it from eIF4E (Ma and Blenis, 2009). S6K include S6K1 and S6K2. It is worth noting that S6K1 has been reported to play a significantly stronger role in the mTORC1 signaling pathway than S6K2 (Fingar et al., 2004; Richardson et al., 2004). S6K are activated by phosphorylation and modulate the interaction between eukaryotic translation initiation factor 4B (eIF4B) and eIF4A, which can enhance the RNA helicase activity of eIF4A and promote the initiation of translation (Ma and Blenis, 2009). In addition, S6K1 has the ability to stimulate ribosomal biosynthesis (Jastrzebski et al., 2007) and can promote the phosphorylation of ribosomal protein S6 (rpS6), an important component of 40S ribosomes (Magnuson et al., 2012; Ruvinsky and Meyuhas, 2006). mTORC1 also has transcriptional effects on various enzymes that contribute to purine synthesis, which will increase the metabolic flux of the de novo purine synthesis pathway in cells, thereby affecting the nucleotide pool that can be used for nucleic acid synthesis (Ben-Sahra et al., 2016).

How then does mTORC1 activity affect cell growth and proliferation? A relatively convincing line of evidence indicates that the activation of eIF4A helicase activity mediated by S6K is crucial for the disassembly of the 5′UTR, which is carried by mRNA and encodes many regulators modulating cell proliferation and growth, such as hypoxia-inducible factor 1α (HIF1α), cyclin D1, fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF) and IGF-1 (Ma and Blenis, 2009).

Autophagy is an intracellular protein degradation process that is conserved in eukaryotes including yeasts and mammals. Under conditions of nutrient starvation, intracellular protein is transported to lysosomes and degraded, and this process is regulated by mTORC1 (Noda, 2017). At least 3 downstream proteins of mTORC1, unc-51-like autophagy-activated kinase 1 (ULK1), autophagy-related gene 13 (ATG13) and transcription elongation factor b (TFEB), have been found, indicating the manner in which mTORC1 directly adjusts autophagy (Hosokawa et al., 2009; Vega-Rubin-de-Celis et al., 2017) (Fig. 2). Atg13, the direct substrate of mTORC1, is a serine-rich protein, which is hyper-phosphorylated under nutrient-rich conditions. When mTORC1 is inhibited, Atg13 is rapidly dephosphorylated with the help of protein phosphatase 2A (PP2A). Atg13 and the 200 kD focal adhesion kinase family interacting protein (FIP200) form a complex with Ulk1. Under nutrient-rich conditions, the Atg13-FIP200-Ulk1 complex associates with mTORC1 through its subunit Raptor, leading to phosphorylation of Ulk1 and Atg13. Conversely, under starvation conditions, Ulk1 is dephosphorylated and separated from mTORC1, leading to autophosphorylation and up-regulation of kinase activity, then phosphorylates Atg13 (Rabanal-Ruiz et al., 2017; Zachari and Ganley, 2017). TFEB is phosphorylated by active mTORC1 under nutrient-rich conditions and is recruited in the cytoplasm. After starvation, dephosphorylated TFEB enters the nucleus and up-regulates some genes related to lysosomes, such as cathepsin and vacuolar H^+-ATPase (V-ATPase) and autophagy-related genes. These are regulated by mTORC1 activity and affect autophagy levels (Nnah et al., 2019).

3. AA signaling upstream of mTORC1

In mammalian cells, AA, especially essential AA (EAA), are not only used as substrates for protein synthesis but also as signal molecules for various signaling pathways, such as the mTORC1 pathway, to promote protein synthesis (Kimball, 2002). Studies have shown that the stimulation of mTORC1 by AA is stronger than that by insulin and other signals (Appuhamy et al., 2011). AA availability, in particular branched-chain AA (BCAA) availability, is so necessary for mTORC1 activity that growth factors and energy cannot activate mTORC1 without AA (Segev and Hay, 2012). Currently, it is generally believed that the main function of the AA signaling upstream of the mTORC1 is to regulate its subcellular localization so that the latter is easily activated by Rheb. It is not clear whether the AA signal itself can directly phosphorylate mTOR.

As a signal node upstream of mTORC1, TSC can interfere with the activation of mTORC1 as the GAP of Rheb, another key player in the mTORC1 signaling pathway. Growth factor and hormone signals phosphorylate TSC2 through the AMPK/Akt pathway. This phosphorylation causes TSC to lose GAP activity, and promotes mTORC1 activation. Either AA availability or growth factor absence can change the subcellular localization of TSC and inhibit mTORC1, demonstrating that TSC is also a target of AA signaling (Demetriades et al., 2016). Another line of research has also shown that AA availability can redistribute TSC2 to the lysosomal surface, which indirectly obstructs mTORC1 from responding to growth factors and energy signals (Demetriades et al., 2014). TSC2-null cells are insensitive to growth factors and energy loss rather than AA loss, implying that AA signaling does not depend on TSC (Smith et al., 2005).

3.1. Lysosome-dependent AA perception: the ‘inside-out’ model

In recent years, researchers have gradually recognized that lysosomal localization is the key to mTORC1 activation by AA. A cell-free system study demonstrated that only a single lysosome structure can satisfy the regulation of mTORC1 by AA (Zoncu et al., 2011). After entering lysosomes, AA can be sensed, and this signal was transmitted in a step-by-step manner through a number of key protein complexes, including V-ATPase, Ragulator and Rag GTPase. This process recruits mTORC1 to the lysosomal membrane where it can be activated by Rheb. Zoncu et al. (2011) named this model of inside to outside perception as the ‘inside-out’ model.

3.1.1. Rag GTPase

The evidence that AA promote mTORC1 to localize to the lysosomal surface came from a study from Sancak et al. (2008, 2010) which suggested that Rag GTPase is a key in this process. Rag is a small GTPase from the Ras superfamily. There are 4 Rag proteins in mammal, namely Rag A, Rag B, Rag C and Rag D. Rag A and Rag B are highly related in sequence and are functionally equivalent, as are Rag C and Rag D (Jewell et al., 2013). In mammalian cells, Rag GTPase exist as a heterodimer involving Rag A or Rag B binding to Rag C or Rag D, and are necessary for mTORC1 activation, as well as their own stability (Sancak et al., 2008) (Fig. 3). The activation state of Rag GTPases is reflected by their guanine nucleotide state, which is regulated by AA signals. In AA-rich conditions, Rag GTPase are in their active form (Rag A/B·GTP - Rag C/D·GDP). Otherwise, this complex widely presents in its inactive form (Rag A/B·GDP - Rag C/D·GTP). After activation, Rag GTPase binds to Raptor and localizes mTORC1 to the surface of lysosome, where mTORC1 kinase can be stimulated by the active Rheb (Sancak et al., 2008). The cryo-electron microscope structure of Rag A/C bound to mTORC1 shows the details of Rag A/C binding to the Raptor subunit of mTORC1. The binding of Rag heterodimer does not change the conformation of mTORC1, but activates it by targeting mTORC1 to the lysosome, which is contrary to the allosteric activation of Rheb (Anandapadamanaban et al., 2019). It was also shown that Raptor has a newly discovered element called a ‘Raptor claw’, which is responsible for recognizing and specifically binding Rag C·GDP

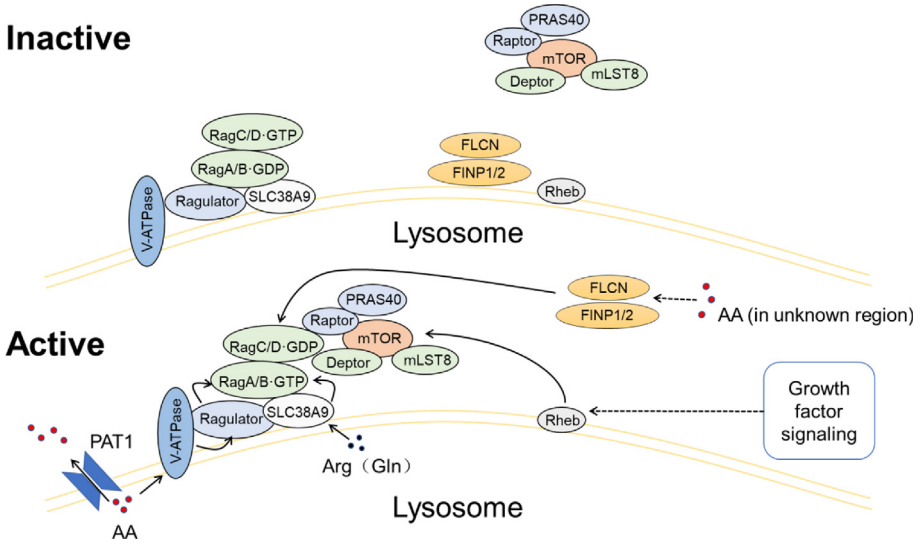

Fig. 3. Lysosomal regulation of mTORC1 by AA: the 'inside-out' model. PRAS40 = 40 kDa Pro-rich AKT substrate; Raptor = regulatory-associated protein of mTOR; mTOR = mammalian or mechanistic target of rapamycin; Deptor = DEP domain-containing mTOR-interacting protein; mLST8 = mammalian lethal with SEC13 protein 8; Rag = Ras-related GTP binding; GDP = guanosine5′-diphosphate; GTP = guanosine 5′-triphosphate; V-ATPase = vacuolar H^+-ATPase; FLCN = Folliculin; FNIP1/2 = folliculin interacting protein 1/2; Rheb = Ras homologue enriched in brain; PAT1 = Proton-assisted Amino acid Transporter 1; AA = amino acid; SLC38A9 = solute carrier family 38 member 9.

instead of Rag C·GTP, linking the activity of Rag GTPase and the lysosomal location of mTORC1 (Rogala et al., 2019). In addition, although Rag A and Rag B, as well as Rag C and Rag D, are homologous and evolutionarily conserved among yeast, insects and advanced mammals, they differ in their expression (Averous et al., 2014; Shimobayashi and Hall, 2016) and function (Dubouloz et al., 2005; Efeyan et al., 2014; Han et al., 2012; Kim et al., 2008). Unexpectedly, Rag GTPase also functions as an inhibitor of mTORC1 in the absence of AA. In AA starvation, S6K1 and 4EBP1 phosphorylation levels in Rag GTPase-deficient mouse embryonic fibroblasts (MEF) are higher than MEF in a control group (Averous et al., 2014). Rag GTPase can sense AA deficiency and recruit TSC2 to the surface of the lysosome, which inhibits the activation of mTORC1 through Rheb (Demetriades et al., 2014; Deng et al., 2015). Moreover, Rag GTPase activity can be controlled by a complex called GAP activity towards Rags (GATOR) (Deng et al., 2015; Jin et al., 2015).

3.1.2. Ragulator

The discovery of Rag GTPase preliminarily demonstrated the manner of AA sensing and mTORC1 activation with lysosomes as the venue. However, it has been shown that the structure of Rag GTPase lacks a lipid-anchoring motif, which means that it cannot be directly localized, but rather should bind to a related protein on the lysosomal membrane (Jewell et al., 2013). Soon, a pentamer (initially thought to be a trimer) called Ragulator was discovered to be an activator of Rag GTPase. It contains p18, also called LAMTOR1, which contains a lipid-anchoring motif. This motif can help Ragulator and its binding partner, Rag GTPase, to bind to lysosomal membranes through palmitoylation and myristoylation (Bar-Peled and Sabatini, 2014; Jewell and Guan, 2013; Sancak et al., 2010; Shimobayashi and Hall, 2016). In addition to LAMTOR1, the components of Ragulator include p14 (LAMTOR2) and MP1 (LAMTOR3), as well as C7orf59 (LAMTOR4) and HBXIP (LAMTOR5) (Bar-Peled et al., 2012). As the guanine exchange factor (GEF) of Rag A/B, Ragulator can also convert the GDP connected with Rag A/B into GTP, subsequently activating the Rag heterodimer (Fig. 3). Deletion of any part of the pentamer ablates the GEF activity of Ragulator, indicating that this GEF works as an intact pentamer rather than a single subunit or trimer (Bar-Peled et al., 2012; Jewell et al., 2013). Even in the absence of control by Rag C/D, it has been shown that Rag A/B•GTP alone is sufficient to activate Rag GTPase, which provides a powerful argument for Ragulator being a key signal protein complex upstream of Rag GTPase (Sancak et al., 2008). In short, Ragulator has been recognized as a key in the AA-mediated mTORC1 signaling pathway, which is located upstream of and activates Rag GTPase by sensing AA availability in the lysosomal lumen (Fig. 3). Strangely, the result of structural analysis shows that there is no known GEF sequence in Ragulator (Bar-Peled et al., 2012). Later studies have proposed a special mechanism of how Ragulator activates Rag GTPase without any GEF structure (Shen and Sabatini, 2018).

3.1.3. V-ATPase

How does Ragulator sense AA availability and where does the upstream signal come from? The answer was given by Zoncu et al. (2011). They demonstrated that V-ATPase may be the key protein complex upstream of Ragulator. V-ATPase, a multi-subunit complex located in the lysosome, divides into 2 domains, V0 and V1. Located inside the lysosomal membrane and containing 5 subunits, the V0 region acts as a channel to pump protons into the lysosome, which help maintain the pH value inside and outside. The V1 region, which contains 8 subunits and localizes outside the lysosome, provides energy for the proton pumping function of the V0 region by hydrolyzing ATP (Nishi and Forgac, 2002). Rag GTPase interacts with V1, and Ragulator interacts with V0 and V1. Both of these interactions are affected by AA availability (Jewell et al., 2013) (Fig. 3). Inhibiting the function of V-ATPase also makes mTORC1 activity insensitive to AA levels (Abu-Remaileh et al., 2017; Zoncu et al., 2011). Moreover, stimulation of starved cells with 14C-labelled AA revealed their rapid recruitment in the lysosome (Zoncu et al., 2011), supporting the 'inside-out' model. A study from Abu-Remaileh et al. (2017) demonstrated that V-ATPase modulates the outflow of many kinds of AA, including arginine (Arg), in a pH-dependent manner, thus affecting mTORC1 activity.

3.1.4. Rheb

The regulatory role of TSC has already been mentioned above. At present, the only identified catalytic domain of TSC2 is its GAP

domain at its C-terminus, which converts Rheb-bound GTP to GDP, decreasing its activation, thus inhibiting mTORC1 (Roccio et al., 2006). Rheb, another small GTPase from the Ras superfamily, was first found in Drosophila. Rheb catalytic activity is determined by the conversion of GTP/GDP, which is regulated by TSC (Yamagata et al., 1994). In mammalian cells, Rheb localizes to various intimal structures, including lysosomes, endoplasmic reticulum (ER), the Golgi, mitochondria and peroxisomes (Melser et al., 2013; Takahashi et al., 2005; Thomas et al., 2014; Zhang et al., 2013). It was reported that Rheb is an important activator of the insulin-mediated mTORC1 signaling pathway. Insulin signal stimulation increased the proportion of Rheb•GTP, and then phosphorylated S6K1 and 4EBP1 in a mTORC1-dependent manner, which could be inhibited by rapamycin (Tabancay et al., 2003; Tzatsos and Kandror, 2006) (Figs. 2 and 3). A recent study showed that Lysosome-anchored E3 ligase RNF152 catalyzes Rheb ubiquitination and promotes its binding to the TSC complex, which is of regulatory importance for the mTOR system (Deng et al., 2019). In terms of AA signaling, although it does not rely on TSC, Rheb participation is still required. When AA levels are sufficient, the proportion of Rheb•GTP in cells increases significantly. Conversely, the activation of Rheb by insulin is blocked when AA are absent (Roccio et al., 2006; Smith et al., 2005). Overexpression of Rheb•GTP makes S6K1 insensitive to AA starvation (Long et al., 2005a, 2005b; Tee et al., 2003). Active Rheb is necessary for the 'inside-out' model because mTORC1 has difficulty being fully stimulated regardless of AA levels or the absence of growth factors.

Understanding of AA signaling in the lysosomal membrane has become gradually clearer due to progress in research over the past few years. Researchers have begun to define mTORC1, Rag GTPase, Ragulator, V-ATPase and Rheb together as a super complex in the lysosome (Yao et al., 2017) and have explored its crystal structure as a whole through the use of a cryo-electron microscopy technology, which is helpful for systematically determining how mTORC1 perceives the availability of AA on the lysosomal membrane. Some related molecular mechanisms have also been revealed through these efforts (Peng and Jewell, 2020). In addition, some key nodes such as Folliculin (FLCN) and its chaperone FINP, as well as Proton-assisted Amino acid Transporter 1(PAT1) and solute carrier family 38 member 9 (SLC38A9), which play signaling roles with this super complex, have also been found. FLCN is an evolutionarily conserved tumor suppressor, whose loss-of-function mutations cause a familial cancer syndrome called Birt-Hogg-Dube' syndrome (BHD) (Nickerson et al., 2002). FINP include FINP1 and FINP2. FLCN-FINP2 senses AA availability and serves as the GAP of Rag C/D, but FLCN-FINP1 seems to have GAP function only for Rag D. It is worth noting that FLCN-FINP1/2 localizes on the lysosomal surface when cells are AA starved. The mechanism of Rag GTPase regulation and the effect of AA availability in response in cytoplasm or the lysosomal lumen is still unclear (Tsun et al., 2013), although recent studies have shown that Leu levels can be maintained in the lysosomal lumen (Wu et al., 2016). PAT1 (or SLC36A1), belonging to the PAT (or SLC36) family, a class of proton-assisted AA transporters, was first found to have a positive regulatory effect on mTORC1 in *Drosophila* (Goberdhan et al., 2005). Based on the results of PAT1 co-immunoprecipitation with Rag GTPase on membranes of late endosomes and the lysosome, Ögmundsdóttir et al. (2012) suggested that PAT1 may participate in mTORC1/lysosome co-localization through interaction with Rag GTPase, Ragulator and V-ATPase. PAT1, whose overexpression can completely inhibit mTORC1 regulation, has the effect of transporting AA out of the lysosome (Zoncu et al., 2011). There is, thus, a functional antagonism between PAT1 and FLCN-FINP1/2 on the surface of the lysosome (Wu et al., 2016). Similar to PAT in structure, SLC38A9 is a member of the AA transporter SLC38 family located on the lysosome. In recent years, SLC38A9 has been thought to transmit Arg signals and activate mTORC1 and also participate in glutamine (Gln) transport (Scalise et al., 2019; Wang et al., 2015). Stable expression of FLAG-labeled SLC38A9 isomer 1 (SLC38A9.1) immunoprecipitates with LAMTOR1/2 (both Ragulator components), and Rags, suggesting that SLC38A9 interacts with them. Additionally, SLC38A9.1 binds to V-ATPase, a transmembrane complex that mediates AA signaling (Wang et al., 2015). Overexpression of SLC38A9.1, or only its Ragulator binding site, makes mTORC1 activation insensitive to AA deletion. This subsequently suggested that SLC38A9 mediates the transporting of various EAA, including Leu, out of the lysosome in an Arg-dependent manner (Scalise et al., 2019). SLC38A9 has no homologue in Drosophila, inferring that its role may not be necessary for the regulation of mTORC1 (Goberdhan et al., 2016). In summary, it has currently been shown that mTORC1 forms a super complex with Rag GTPase, Ragulator and V-ATPase in the lysosome, and FLCN-FINP1/2, PAT1 and SLC38A9 cooperate with this complex to respond to AA signaling (Fig. 3). Furthermore, SLC38A9 seems to be a specific Arg sensor and most likely directly senses Arg levels.

mTOR appeared before both the insulin receptor and PI3K (Avruch et al., 2006). Moreover, in the evolution of single-celled organisms to mammals, the AA signaling upstream of mTORC1 has certainly changed. The high homology of Gtr and Rag GTPase implies the selectivity of organism evolution. Rag A and B are incredibly close in function and sequence (the same is true for Rag C and D). What, then, is the evolutionary meaning of the 'one divides into two'? This fundamental question has not been thoroughly answered, though many studies have shown that there are functional and expression differences between Rag A and B (or Rag C and D) (Efeyan et al., 2013, 2014; Han et al., 2012; Sancak et al., 2008). It has been currently proposed that in mammals, mTORC1 is recruited to the lysosome in response to AA availability using the 'inside-out' mechanism, whereas AA depletion releases mTORC1 to the cytoplasm or unknown regions, which differs from single-celled organisms. For example, in yeast, TORC1 is always located on the surface of vesicles, which are functionally similar to mammalian lysosomes, and does not respond to AA availability (Binda et al., 2009). Rheb plays a key role in catalyzing mTORC1 in response to changes in growth factors such as insulin, and energy states in advanced mammalian cells (Avruch et al., 2006; Roccio et al., 2006; Tabancay et al., 2003; Tee et al., 2003). In single-celled organisms, however, there is no PI3K or insulin, so Rheb does not have similar functions. mTORC1 integrates AA, glucose, growth factors, stress and other input signals, regulates anabolism, including protein and lipid synthesis, as well as catabolism, such as autophagy. Cell growth and proliferation involve crucial processes, such as translation, which require a large amount of energy (Shimizu et al., 2001). These must have sufficient raw materials and power to drive protein synthesis in order to ensure normal growth and proliferation, which may prompt mammals to evolve finer sensory mechanisms (Ma and Blenis, 2009). The change from Gtr1/2 to Rag GTPase doubled the number of sites that can receive these signals, which meant that the types of upstream signals can be diversified more accurately than before. This contributed to integrating these sophisticated network signals and choosing the most appropriate cellular status. The emergence of hormones enabled organs or tissues to convey information to each other and to make physiological adjustments according to their external environment. Thus, it was significant that Rheb evolved as a growth factor target to receive such signals and transmit them to mTORC1 over evolutionary time. The lysosomal localization of mTORC1 determines its activation and must be determined by both AA availability and growth factor signals. AA availability is responsible for localizing mTORC1 to the lysosomal surface, while growth factors activate

mTORC1 through Rheb, both of which are indispensable (Wang et al., 2015). This 'double insurance' is beneficial for mammal's adaptations to different environments and also avoids the uncontrolled mTORC1 activity caused by unilateral disordered inputs. These 2 signals have also been shown to have synergistic effects in activating mTORC1 (Avruch et al., 2006; Roccio et al., 2006; Yoon et al., 2020). However, notwithstanding, as sufficient evidence has not been found to demonstrate this yet, these evolutions from simple to complex have considerable practical significance.

3.2. Sensing AA in the cytoplasm through GATOR and Leucyl-tRNA synthetase

The current progress on the 'inside-out' model, which shows that Rag GTPase locates on the lysosomal membrane, converging on multiple signals to sense the AA level in the lysosomal lumen has been described above. In addition, lysosomal AA have regulatory effects on mTORC1. Studies of leucyl-tRNA synthetase (LRS) and GATOR have revealed that mTORC1 activation can respond to free AA in the cytoplasm.

3.2.1. GATOR

The discovery of GATOR stems from the identification of proteins from the co-precipitation of Rag by Bar-Peled et al. (2013). GATOR contains 8 proteins and acts as a GAP for Rag A/B. It is a multimeric complex composed of GATOR1 and GATOR2. GATOR1 is a trimer composed of DEP domain-containing protein 5 (DEPDC5), Nprl2 and Nprl3, and the GATOR2 pentamer consists of Mios, WDR24, WDR59, Seh1L and Sec13 (Bar-Peled et al., 2013). The latest research suggests that Arg78 on Nprl2 is a key for GATOR1's GAP function (Shen et al., 2019). CUL3-KLHL22, an E3 ubiquitin ligase, seems to promote the degradation of DEPDC5, a component of GATOR1, which is regulated by AA and is conserved across evolution (Chen et al., 2018). The modulation of mTORC1 by GATOR1 is upstream of Rag GTPase. Sensing the AA depletion, GATOR1 can localize to the surface of lysosomes, then bind Rag A/B and acts as their GAP to inhibit the lysosomal localization and activation of mTORC1, which is the opposite of the function of Ragulator. On the contrary, responding to AA availability, GATOR2 can combine with GATOR1 and inhibit its GAP function, rather then positively controlling mTORC1 (Bar-Peled et al., 2013; Fingar, 2015) (Fig. 4). The discovery of GATOR was very meaningful. First, it made up for the shortcomings of previous models, which lacked key upstream signals for the negative control of Rag GTPase. Second, it opened up a new research direction upstream of Rag-Raptor. Subsequent studies have shown that various key signaling proteins or complexes, such as RNF152, Skp2 and Sestrins, can affect Rag GTPase and mTORC1 by GATOR.

3.2.2. RNF152 and Skp2

The discoveries of RNF152 and Skp2 were almost simultaneous. RNF152 promotes TSC2 binding to Rag A, which helps in TSC lysosomal localization, and negatively regulates mTORC1. Coincidentally, Skp2 also binds to Rag A and inhibits mTORC1 (Deng et al., 2015; Jin et al., 2015) (Fig. 4). Roles of RNF152 and Skp2 have many similarities. For example, they both need the participation of GATOR1, both act on Rag A and inactivate it through polyubiquitination, both recruit TSC to lysosomal membranes, and most importantly, both are regulated by the availability of AA (Fingar, 2015). The difference between RNF152 and Skp2 is that they are activated under different conditions. RNF152 is thought to be initiated during intracellular AA withdrawal, whereas the role of Skp2 has been shown to prevent the over-activation of mTORC1 in the case of long-term AA saturation (Fingar, 2015).

3.2.3. SAMTOR and CASTOR

SAMTOR and CASTOR are receptors of cytoplasmic Met and Arg, respectively. In addition to RNF152 and Skp2, other GATOR regulators have been identified in recent studies. KICSTOR, a tetramer composed of KPTN, ITFG2, C12orf66 and SZT2, localizes to the lysosomal surface and recruits GATOR1, which responds to a loss of glucose and AA (Wolfson et al., 2017). A subsequent study focused on the role of SZT2 (Peng et al., 2017). Regarding KISCTOR, Gu et al. (2017) further identified a previously unreported protein called SAMTOR. SAMTOR combines with KICSTOR and GATOR1 to form a super complex, promoting the GAP activity of GATOR1 and inhibiting the interaction between GATOR1 and GATOR2. SAMTOR indirectly senses the availability of cytoplasmic Met by binding to S-adenosylmethionine (SAM), a type of Met metabolite. After binding SAM, SAMTOR promotes the activation of mTORC1 through disassociating itself from GATOR1 (Gu et al., 2017). In summary, SAMTOR is a Met receptor that indirectly senses cytoplasmic Met availability through SAM and regulates mTORC1 activity through GATOR1 and KICSTOR (Fig. 4). However, Arg seems to have a special

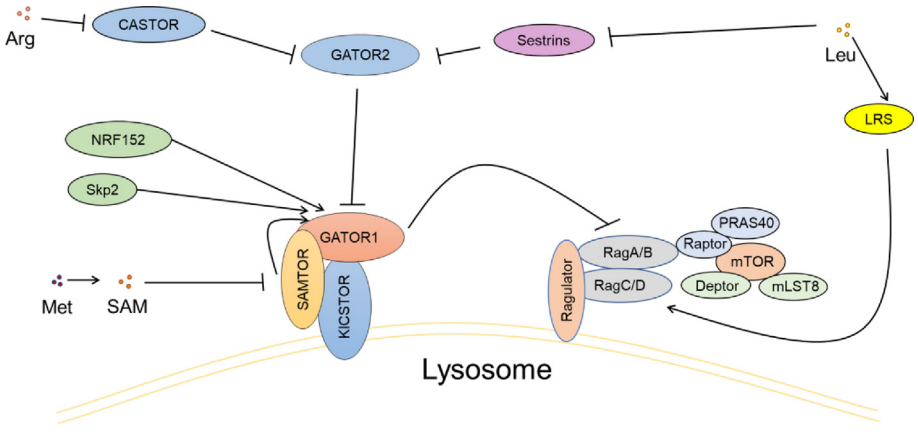

Fig. 4. Cytoplasmic AA-sensing pathway upstream of mTORC1. CASTOR = cellular arginine sensor for mTORC1; GATOR2 = GAP activity towards Rags 2; GATOR1 = GAP activity towards Rags 1; SAM = S-adenosylmethionine; SAMTOR = S-adenosylmethionine sensor upstream of mTORC1; KICSTOR = Kaptin (KPTN), Integrin alphaphenylalanyl-glycyl-glycyl-alanyl-prolyl (FG-GAP) repeat containing 2 (ITFG2), chromosome 12 open reading frame 66 (C12orf66) and seizure threshold 2 homolog (SZT2)-containing regulator of mTORC1; LRS = leucyl-tRNA synthetase; Rag = Ras-related GTP binding; GDP = guanosine5′-diphosphate; GTP = guanosine 5′-triphosphate; PRAS40 = 40 kDa Pro-rich AKT substrate; Raptor = regulatory-associated protein of mTOR; mTOR = mammalian or mechanistic target of rapamycin; Deptor = DEP domain-containing mTOR-interacting protein; mLST8 = mammalian lethal with SEC13 protein 8.

regulatory manner for GATOR2. Separate from SLC38A9, it was recently reported that CASTOR is also an Arg sensor that senses a lack of cytoplasmic Arg and completes the activation of GATOR1 by inhibiting GATOR2 (Chantranupong et al., 2016; Saxton et al., 2016) (Fig. 4). CASTOR1 and CASTOR2, appearing in the form of a homodimer (CASTOR1-CASTOR1) or heterodimer (CASTOR1-CASTOR2), were found through identification by co-immunoprecipitation and mass spectrometry in research by Chantranupong et al. (2016). CASTOR2 has a higher affinity with GATOR2 than CASTOR1, but it does not respond to Arg availability (Wong, 2016).

3.2.4. LRS and Sestrins

LRS and Sestrins are receptors for leucine. Lately, EAA, especially BCAA, have received extensive attention due to their strong stimulus signal in the mTORC1 signal pathway. The BCAA Leu has been recognized as the most effective AA in mTORC1 activation in a large number of studies (Averous et al., 2014; Beugnet et al., 2003; Fox et al., 1998; Hara et al., 1998). Researchers have also made new discoveries about the Leu signaling pathway, which include the interactions of LRS and Rag D as well as Sestrins and GATOR2. As an aminoacyl-tRNA synthetase, LRS can catalyze the aminoacylation of $tRNA_{LEU}$. That is, it can sense the level of intracellular Leu and promote the charge to its corresponding tRNA. Han et al. (2012) found that LRS has the additional function of binding intracellular Leu, then activating Rag GTPase by combining with Rag D and acting as its GAP, which promotes the lysosomal localization of mTORC1 (Fig. 4). Another study demonstrated that LRS is a Leu biosensor upstream of Vps34 (a class III PI3K)-PLD1 in the mTORC1 signaling pathway by controlling PLD1's lysosomal localization and activation (Yoon et al., 2016). In a recent study, it was shown that the interaction between LRS and Rag D can be constrained by low energy (Yoon et al., 2020). Regarding Sestrins, they are not newly identified. As early as 1999, Sestrins (Sestrin1/2/3) have been seen as a tumor suppressor suppressing p53 transcription activity target in the public eye (Velasco-Miguel et al., 1999), and have been continuously shown to have a negative effect on mTORC1 (Chantranupong et al., 2014; Peng et al., 2014). Budanov and Karin (2008) demonstrated that Sestrin1 and Sestrin2, the products of 2 p53 target genes, activate AMPK, target it to phosphorylate TSC2, stimulate its GAP activity, thereby inhibiting mTOR, step by step. The study demonstrating Sestrins to be a direct receptor for Leu came from Wolfson et al. (2016). They suggest that adding Leu instead of Arg promoted the dissociation of Sestrin1 (or Sestrin2) and WDR24 (a component of GATOR2). Sestrin2 dissociates GATOR2 from GATOR1 and loses its inhibitory effects, whereas Leu availability can cancel it (Fig. 4). LRS and Sestrins in the Rag GTPase–mTORC1 pathway have positive and negative roles, respectively, acting as 'ON' and 'OFF' switches, which both oppose and complement each other (Lee et al., 2018).

3.3. AA sensing by Rab1A at the Golgi

It has been proposed that Rheb is expressed at some intimal structures, including the lysosome and the Golgi. Coincidentally, according to the latest research, mTORC1 can also localize to the Golgi and be activated by Rheb there. Another small GTPase, Rab1A, which lies on the Golgi, can mediate AA signaling independently of Rag A/B (Thomas et al., 2014). With sufficient AA, GTP-bound Rab1A, which is the active form, can recruit mTORC1 and help Rheb activate it. Overexpression of Rab1A promotes mTORC1 signal transduction and carcinogenic growth in an AA-dependent manner (Thomas et al., 2014) (Fig. 5). Sanchez-Gurmaches and Guertin (2014) explained in detail in their review, where they believed that Rab1A functions in an AA sensing pathway distinct from the Rag pathway that is either redundant or senses a different AA pool, but whether these 2 mechanisms work in parallel or are part of contiguous endomembrane-based mTORC1 regulatory system remains to be seen. Rab1A is often overexpressed in hepatocellular carcinoma (HCC), thereby enhancing the overactive AA mTORC1 signal and promoting the malignant growth and metastasis of HCC in *vitro* and in *vivo*. Therefore, Xu et al. (2015) believe that Rab1A is an important biomarker for liver cancer prognosis and personalized mTORC1 targeted therapy. It also promotes proliferation and migration abilities via regulation of the mTOR/S6K1 pathway in colorectal cancer (Cheng et al., 2019). In addition, PAT4 (SLC36A4), a Golgi-based AA transporter, is thought to interact with mTORC1 and Rab1A at the Golgi apparatus (Fan et al., 2016) (Fig. 5). While lysosomal-located mTORC1 senses AA on lysosomes, mTORC1 located on the Golgi may be regulated by AA trafficked back in a retrograde fashion from the endosomal system or pumped into the Golgi lumen by transporters (Goberdhan et al., 2016). This discovery of an mTORC1 activation mechanism provides a powerful tool for understanding the mTORC1 signaling regulated by AA.

3.4. Gln regulation of mTORC1

Gln has been shown to regulate mTORC1 by affecting autophagy, transamination and other specific functions (Jewell et al., 2015; Scott and Klionsky, 1998; Tan et al., 2017) (Fig. 6). Autophagy is an evolutionarily conserved cellular process. Under starvation conditions, proteins and some substances are encapsulated in autophagosomes, and then transferred to the lysosome for degradation. These processes release substances, including AA, that can be used to synthesize key proteins to sustain life (Scott and Klionsky, 1998). AA produced by autophagy can recover mTORC1 signaling and stop autophagy, forming a complete feedback loop (Yu et al., 2010). Gln enters the cell through SLC1A5, and then rapid outflows by SLC7A5/SLC3A2 achieve bidirectional transport with extracellular Leu (Nicklin et al., 2009). In a long-term lack of AA, Gln can supplement the non-essential AA (NEAA) pool through transamination and restore partial activity to mTORC1 (Tan et al., 2017). In another study, it was suggested that Gln could up-regulate AA transporters and provide more EAA from outside to restore mTORC1 activity through the general AA control (GAAC) pathway (Chen et al., 2014). α-ketoglutarate, a kind of Gln metabolite, can activate Rag-mTORC1,

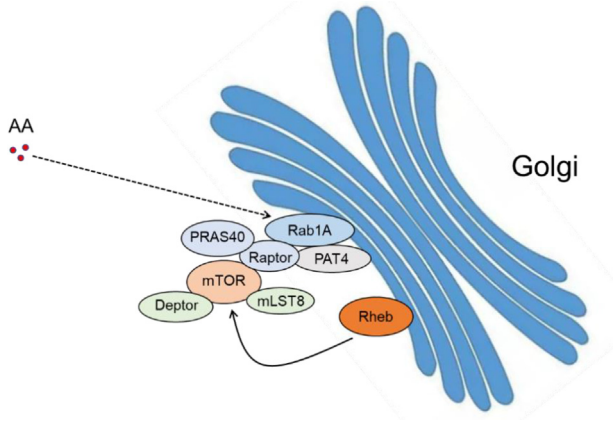

Fig. 5. AA sensing from the Golgi. The location of AA and the mechanism of sensing by Rab1A are still unclear, so the dotted line is used here. AA = amino acid; PRAS40 = 40 kDa Pro-rich AKT substrate; Raptor = regulatory-associated protein of mTOR; mTOR = mammalian or mechanistic target of rapamycin; Deptor = DEP domain-containing mTOR-interacting protein; mLST8 = mammalian lethal with SEC13 protein 8; Rab1A = Ras related protein Rab 1A; PAT4 = phytochrome A signal transduction 4; Rheb = Ras homologue enriched in brain.

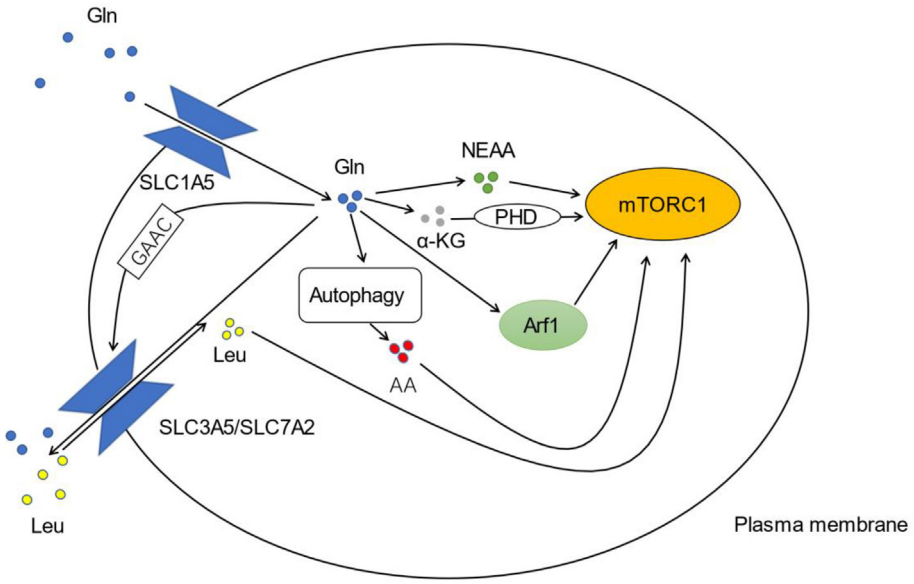

Fig. 6. Regulation of mTORC1 activation by Gln. SLC1A5 = solute carrier family 1 member 5; NEAA = non-essential AA; mTORC1 = mechanistic target of rapamycin complex 1; GAAC = general amino acid control; α-KG = α-ketoglutarate; PHD = prolyl hydroxylase; Arf1: ADP ribosylation factor 1; SLC3A5/SLC7A2: solute carrier family 3 member 5/solute carrier family 7 member 2; AA = amino acid.

which may occur through prolyl hydroxylase (PHD) (Durán et al., 2012; Villar et al., 2015). Apart from autophagy and the GAAC pathway, Gln has its specific 'sensor', the ADP ribosylation factor 1 (Arf1), which mediates Gln signaling and activates mTORC1 in a form independent of Ragulator and Rag GTPase (Jewell et al., 2015). Interestingly, Arf1 is widely localized to the Golgi apparatus, which also provides another potential option for the activation of mTORC1 on Golgi (Carroll, 2020).

3.5. Potential extracellular AA sensing pathways

Although mechanisms of AA sensing in cells (especially on the lysosome and cytoplasm) have been continuously demonstrated, it is well known that mammals can also perceive the availability of extracellular AA. First of all, in studies in vitro, we often observe that mTORC1 activation can recover in response to supplementation within a few minutes after long-term AA starvation, despite the AA transport being limited over such a short period of time, which is not enough to activate mTORC1. Therefore, it is possible that the AA levels of the external fluid can be sensed and preliminary responses to mTORC1 activation can be prepared. Second, most of the studies used the method of removing all AA, that is, in vitro experiments on AA starvation treatment. However, in mammals, even if starved for a long time, plasma AA levels will not drop as significantly as in vitro (Stegink et al., 1991). This makes us wonder whether the subcellular localization of mTORC1 can be inhibited by the absence of AA in vivo. Based on this, Bohé et al. (2003) determined that there was indeed a perception of extracellular AA in mammals, which affects mTORC1 activation and even protein synthesis in muscle. Skeletal muscle cells have the inherent ability to sense mechanical signals and convert this information into biochemical events that regulate growth (Hornberger et al., 2006). Goodman (2014) proposed that the mTORC1 signal is necessary for protein synthesis and muscle mass changes in response to exercises such as overload/resistance, endurance, and sprints. By the way, many molecules and nodes, such as IGF-1, ERK1/2, PA, TSC, AA, as well as metal ions, as Ca^{2+}, seem to play important roles in the activation of mTORC1 signaling in response to mechanical stimulation (Goodman, 2014; Hornberger, 2011). Several studies on G protein-coupled receptors, such as the heterodimers T1R1-T1R3 (Wauson et al., 2012) and GPRC6A (Fujiwara et al., 2014), have shown some mechanisms for the extracellular AA perception of mammals, although their details are still not completely clear. In addition, mammals can, through extracellular Ca^{2+} sensing receptors (Conigrave et al., 2000), utilize appetite (Cummings and Overduin, 2007) and hormones (Kim and Guan, 2011) to sense extracellular AA.

3.6. Differences in the regulation of mTORC1 by AA

3.6.1. Stimulation intensities of mTORC1 by individual AA

There are hundreds of types of AA in nature, but only around 20 kinds can be used in protein biosynthesis. According to whether they can be generated in mammals, these are divided into EAA and NEAA. Generally speaking, the activation of mTORC1 is more dependent on EAA, which can only be absorbed from the outside, rather than NEAA, which can be synthesized in cells (Hara et al., 1998). As early as the 20th century, studies have shown that different AA have different stimulation intensities for mTORC1 (Wang et al., 1998; Zhou et al., 2016). At present, it is generally believed that Leu and Arg are the strongest stimulators of mTORC1. For example, in the study from Hara et al. (1998), the inhibitory effect of the depletion of Leu or Arg on mTORC1 was similar to depletion of all AA. It has also been reported that other BCAA such as isoleucine (Ile) and valine (Val) also seem to have strong activation capacities (Appuhamy et al., 2012; Herningtyas et al., 2008). In some specific tissues, other EAA such as Met and Thr have strong stimulatory effects (Ryu and Han, 2011; Zhou et al., 2016). As progress has continued, Gln has gradually attracted our attention in mTORC1 signaling pathways (Chen et al., 2014; Jewell et al., 2015; Tan et al., 2017). In fact, there is increasing evidence that specific AA, such as BCAA and Arg, are stronger stimulators on mTORC1 signal than the others. Later studies revealed that these AA stimulate mTORC1 signal through specific pathways. AA are the blocks for protein synthesis in living organisms, and their respective intracellular concentration is generally sensed by their specific aminoacyl-tRNA syntheses. It is unrealistic and uneconomical to sense and summarize the concentration of each AA, obviously. It is

reasonable to prioritize the detection of certain representative AA. First, take Leu as an example, it is the most frequently used AA in proteins, and LRS is one of the most abundant proteins expressed in yeast; secondly, under natural conditions, it is uncommon for some AA to be overtaken and others to be completely absent, that is, if cells have a sufficient supply of leucine, chances are high that the other AA are present as well (Segev and Hay, 2012).

3.6.2. Differences in AA signaling between cell types

As of today, extensive studies have been carried out both *in vitro* and *in vivo* on human and many other mammals (mouse, rat, ruminant, pig, and so on), demonstrating that different tissues respond dissimilarly to AA signals by mTORC1. In a study of a low-protein diet supplemented with Leu to piglets, Leu increased mTORC1 activity and protein synthesis efficiency in skeletal muscle and most internal organs, but not in the liver (Murgas Torrazza et al., 2010). We also found that the sensitivity of dairy cow mammary glands to Leu was not as high as previously observed in human muscle. In other words, the inhibitory effect of Leu starvation on mTORC1 was much lower than that from removing all of the EAA. The stimulation intensity of Ile seemed to be similar to Leu, which was likely due to the characteristic of breast tissues compared with other tissues (Appuhamy et al., 2012; Arriola Apelo et al., 2014; Liu et al., 2017; Moshel et al., 2006). It has been previously reported that Ile may have a similar ability to increase milk protein synthesis as Leu (Richert et al., 1997). This may be caused by the particularity of mammary glands. Milk is a unique evolutionary characteristic of mammals, which can ensure orderly postpartum programming to regulate individuals to maintain proper growth rates. In other words, it represents a highly complex endocrine signaling system, not just a 'simple food'. This is beneficial for newborns to activate and maintain mTORC1-dependent translation and other mTORC1-mediated anabolic effects during growth. This may require the mammary gland to regulate protein synthesis in a

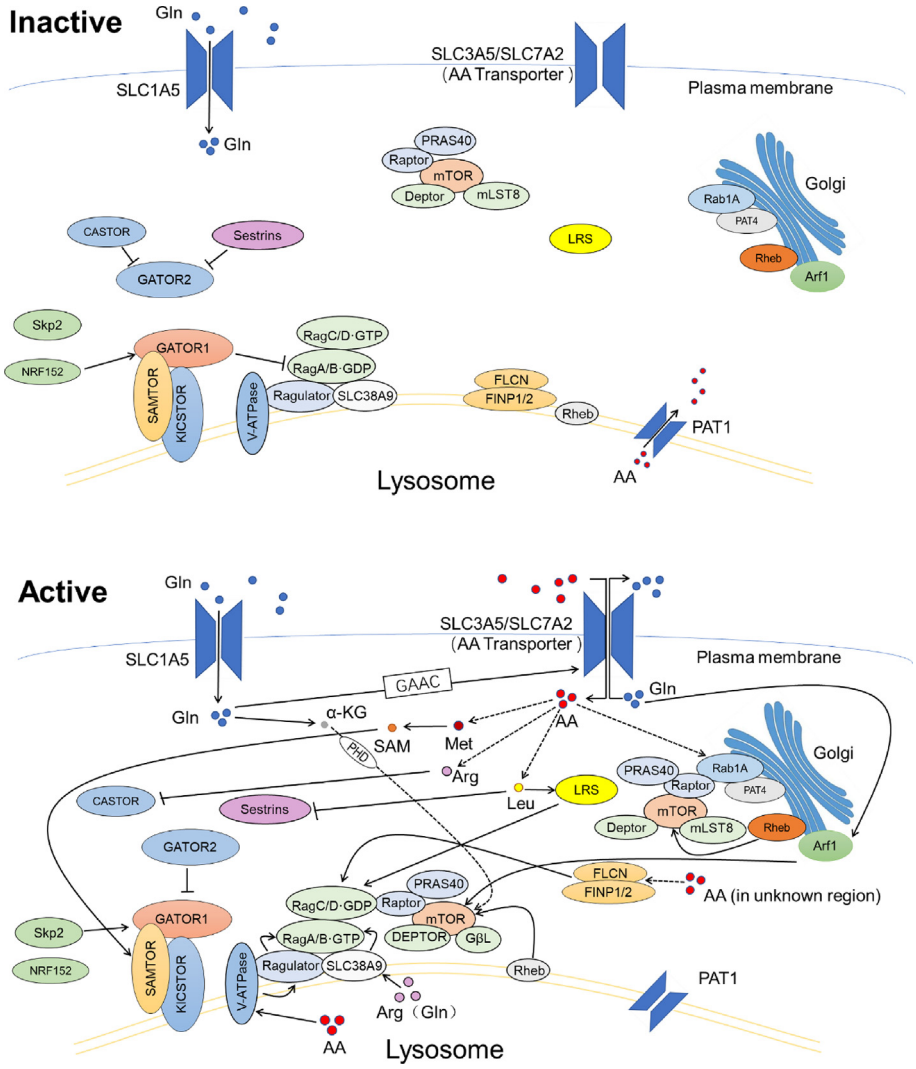

Fig. 7. Regulation of mTORC1 by AA in mammalian cells. SLC1A5 = solute carrier family 1 member 5; SLC3A5/SLC7A2: solute carrier family 3 member 5/solute carrier family 7 member 2; GAAC = general amino acid control; α-KG = α-ketoglutarate; PHD = prolyl hydroxylase; SAM = S-adenosylmethionine; AA = amino acid; CASTOR = cellular arginine sensor for mTORC1; LRS = leucyl-tRNA synthetase; RAS40 = 40 kDa Pro-rich AKT substrate; Raptor = regulatory-associated protein of mTOR; mTOR = mammalian or mechanistic target of rapamycin; Deptor = DEP domain-containing mTOR-interacting protein; mLST8 = mammalian lethal with SEC13 protein 8; Rab1A = Ras related protein Rab 1A; PAT4 = phytochrome A signal transduction 4; Rheb = Ras homologue enriched in brain; Arf1: ADP ribosylation factor 1; GATOR2 = GAP activity towards Rags 2; GATOR1 = GAP activity towards Rags 1; SAMTOR = S-adenosylmethionine sensor upstream of mTORC1; KICSTOR = Kaptin (KPTN), Integrin alphaphenylalanyl-glycyl-glycyl-alanyl-prolyl (FG-GAP) repeat containing 2 (ITFG2), chromosome 12 open reading frame 66 (C12orf66) and seizure threshold 2 homolog (SZT2)-containing regulator of mTORC1; V-ATPase = vacuolar H+-ATPase; Rag = Ras-related GTP binding; GDP = guanosine5′-diphosphate; GTP = guanosine 5′-triphosphate; SLC38A9 = solute carrier family 38 member 9; Rheb = Ras homologue enriched in brain; FLCN = Folliculin; FNIP1/2 = folliculin interacting protein 1/2; PAT1 = Proton-assisted Amino acid Transporter 1.

different way than other organs to adapt to its unique physiological functions (Melnik, 2015). Another possible explanation is in cells whose mTORC1 activation was affected by multiple AA, the mechanism of mTOR activation may differ from cells whose mTORC1 activation was mainly regulated by Leu (Lynch, 2001). Some cells, such as hepatoma cell lines, have strong resistance to AA removal, and Shigemitsu et al. (1999) suggested this may be caused by a higher rate of endogenous autophagy. These findings may also be due to inter-species variability, but we hold the opinion that it is more likely due to organizational differences. First, most tissues (or organs) have their own division of labor, which may lead to discrepancies in AA uptake. Take mammary glands as an example. When the supply of EAA decreases, mammals usually choose to increase the blood flow of the mammary vein to obtain sufficient raw materials for milk protein synthesis (Bequette et al., 2000; Liu et al., 2019), which is not a common phenomenon in other organizations. Second, the expressions and activities of AA transporters differ between cells, the same as proteins involved in the mTORC1 pathway, which also leads to varying results. Finally, most of these results came from the respective research teams, and the errors caused by the use of serum components in *vitro* experiments, and by the compositions of diets in *vivo* experiments, are inevitable.

4. Discussion

Here, we refer to previously proposed ideas (Bond, 2016; Goberdhan et al., 2016; Peng and Jewell, 2020; Shimobayashi and Hall, 2016; Tan et al., 2017; Zoncu et al., 2011) and integrate them together (Fig. 7). mTORC1 may require the availabilities of various AA in the lysosomal lumen and cytoplasm, as well as on the Golgi, rather than a single availability. Rag GTPase seems to be crucial, first of all, as the activation of Rag A/B needs to sense lysosomal AA through the 'super complex' and SLC38A9, and then cytosolic AA can release GATOR from Rag A/B. Additionally, Leu can combine and affect Rag C/D through LRS. It is worth noting that although mTORC1 signaling can be activated by the lysosome alone in a cell-free system, GATOR and other potential proteins or complexes that inhibit mTORC1 may not be complete in this system. In addition, we have seen that Arf1 and Rab1A on the Golgi mediate AA signaling in each Rags-independent way, which can be used as a supplement to this main route. Gln restores mTORC1 activity in many situations (especially during AA starvation) in a variety of independent ways. Here, we integrate them as much as possible. Some issues have not been resolved through this effort, though. For example, why AA must be sensed in different parts to regulate mTORC1 and where AA that are sensed by FLCN-FINP1/2 come from.

We suggest that cells can sense signals from different AA at multiple nodes and eventually converge them to mTORC1. Lysosomes may be the primary sensing site, which has been proven in many studies. They are the so-called 'AA pool' in cells and undertake the final step of autophagy, which means that AA produced by protein degradation is recruited here. As a supplement, cells could sense part of the AA in the cytoplasm, which helps them perceive and respond to changes in the availability of AA in time. As the most important NEAA, Gln is the main regulator of the cell NEAA pool, which regulates mTORC1 activity in the above-mentioned ways, as seems logical. The reason for the perceived availability of AA on the Golgi apparatus is not yet clear. Maybe this avoids excessive dependence on Rag GTPase to a certain extent. The controllability of mTORC1 signaling is essential to the health of mammals as well as other animals, but Rag GTPase is not evolutionarily conserved, as mentioned above. In addition, we should probably analyze the reason from the physiological function of the Golgi in protein modification, though there is currently no sufficient evidence. To sum up, these all require more research to confirm.

Sensing AA signals at different nodes may have different meanings. Take Leu, for example. In most cases, it may mainly regulate mTORC1 activity through the 'super complex' on lysosomes and LRS. When AA is insufficient, Gln exerts its regulatory function to partially restore mTORC1 activity to maintain cell survival, and accelerates the intake of Leu and other AA through the bidirectional transport mechanism. At this time, cells may prepare for the reactivation of mTORC1 in a special way, which is why AA-starved cells can recover mTORC1 activity in just a few minutes after supplementing with AA. Under some specific conditions, Sestrins can be activated and inhibit the mTORC1 signaling pathway, whereas Leu can quickly dissociate it from GATOR 2 by directly binding to it. Extensive and sufficient research is still needed to prove whether it is so.

5. Conclusion

Since the dawn of the 21st century, much progress has been made in research on the AA signals upstream of the mTORC1 pathway. Researchers have identified key proteins, such as Rag GTPase and Ragulator, observed the role of AA transporters, such as PAT and SLC38A9 on this signal pathway, and even found 2 Leu direct receptors, LRS and Sestrins, as well as the Arg receptor CASTOR and the Met indirect receptor SAMTOR. Currently, the lysosome is considered to be the main AA sensing site, where Rag GTPase integrate signals from multiple pathways such as those sensed by V-ATPase and GATOR (Bar-Peled et al., 2013; Fingar, 2015). AA (such as Leu, Arg, Met and Gln) can also be sensed in the cytoplasm and act on different sites through LRS, Sestrins, Arf1, and so on (Jewell et al., 2015; Lee et al., 2018). In addition, Rab1A, another small GTPase located on the Golgi apparatus, can transmit AA signals independently of Rag GTPase (Thomas et al., 2014). Now, we collate the current research progress to summarize the current scattered possible pathway mechanisms for observation. In addition, different species and cell types should be considered, because current research hints that any pathway model may not be applicable to all of them.

There are still many problems that require an urgent solution. For example, the mechanism of V-ATPase and GATOR2 have not been fully demonstrated. The significance of the evolution of yeast Gtr1/2 with Rag GTPase from mammals is also unclear. In addition, it is important to note that there are differences in AA signals in different cells, while if not clearly explained, was discussed above. The exploration of these problems will help us understand the different needs of various organizations for AA. The potential receptors of Leu, Arg and Met have been found, which may explain why we have observed that their starvation has a strong inhibitory effect on mTORC1. Interestingly, Thr and other AA are also involved in the mTORC1 signaling pathway (Ryu and Han, 2011), but the specific reason is unknown. Both LRS and Sestrins have a low affinity for Ile (Han et al., 2012; Wolfson et al., 2016), whereas Ile has been shown to have the same ability as Leu to activate mTORC1 in cattle mammary glands (Appuhamy et al., 2012; Liu et al., 2017), it is still not clear whether there is a connection between them. Potential receptors of other AA also await discovery in the future.

Author contributions

Shizhe Zhang: Conceptualization, Writing- Original draft preparation. **Xueyan Lin:** Writing - Review & Editing, Supervision. **Qiuling Hou:** Writing - Review & Editing. **Zhiyong Hu:** Writing -

Review & Editing. **Yun Wang:** Writing - Review & Editing. **Zhonghua Wang:** Funding acquisition, Supervision.

Conflict of interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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